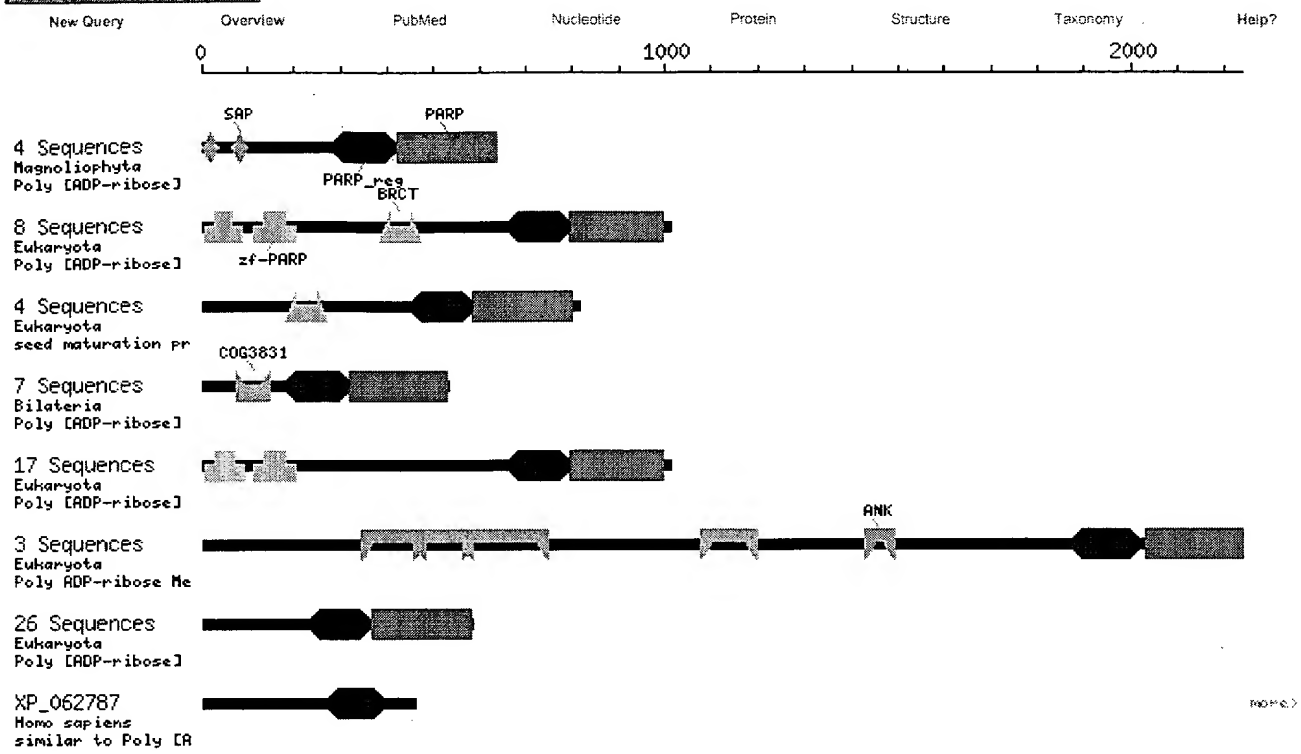
Exhibit I 09/843159

CDART: Conserved Domain Architecture Retrieval Tool



Result page: Previous 1 Next

Subset by Taxonomy

Subset by selected domains:

☐	pfam00644	Poly(ADP-ribose) polymerase catalytic domain. Pol...
☐	COG3831	Uncharacterized conserved protein [Function unkno...
☐	cd00204	ankyrin repeats; ankyrin repeats mediate protein...
	includes:	COG0666 COG3779
☐	pfam00533	BRCA1 C Terminus (BRCT) domain. The BRCT domain i...
	includes:	smart00292 cd00027
☐	pfam02037	SAP domain. The SAP (after SAF-A/B, Acinus and PI...
	includes:	smart00513
☑	pfam02877	Poly(ADP-ribose) polymerase, regulatory domain. P...
☐	pfam00645	Poly(ADP-ribose) polymerase and DNA-Ligase Zn-fin...

Exhibit J 09/843,159 1/2

NCBI

Conserved Domain Database

PubMed Nucleotide Protein Structure CDD Taxonomy Help?

CD: pfam02877.8, PARP_reg **PSSM-Id:** 3371 **Source:** Pfam[US], Pfam[UK]

Description: Poly(ADP-ribose) polymerase, regulatory domain. Poly(ADP-ribose) polymerase catalyses the covalent attachment of ADP-ribose units from NAD+ to itself and to a limited number of other DNA binding proteins, which decreases their affinity for DNA. Poly(ADP-ribose) polymerase is a regulatory component induced by DNA damage. The carboxyl-terminal region is the most highly conserved region of the protein. Experiments have shown that a carboxyl 40 kDa fragment is still catalytically active.

Taxa: Eukaryota **References:** 3 Pubmed Links

Status: Alignment from source **Created:** 11-Apr-2003

Aligned: 6 rows **PSSM:** 134 columns **Representative:** Consensus

Proteins: [Click here for CDART summary of Proteins containing pfam02877]

View 3D Structure with Cn3D using Virtual Bonds (To display structure, download Cn3D)

View Alignment as Hypertext width 60 color at 2.0 bits

Subset Rows up to 10 of the most diverse members

```
                      10        20        30        40        50        60
                 ....*....|....*....|....*....|....*....|....*....|....*....|
consensus      1 KSNLLKSVQDLIKLIFDVDSMAQTMMEFEI--DMEKMPLGKLSKRQIQSAYRVLKEIYEV  58
3PAX           9 KSKLAKPIQDLIKMIFDVESMKKAMVEFEI--DLQKMPLGKLSKRQIQSAYSILNEVQQA  66
gi 1353140   171 LLKQLK-FNEAFGRPIDCLSLAQLTTGYEIlsKIEESIGGKSARRSTRGRPRVADRVLAV  229
gi 1709740   286 QSKLDTRVAKFISLICNVSMMAQHMMEIGY--NANKLPLGKISKSTISKGYEVLKRISEV  343
gi 548585    644 TSKLEISVQNLIKLIFDIDSMNKTLMEFHI--DMDKMPLGKLSAHQIQSAYRVVKEIYNV  701
gi 1709741   647 KSNLPLSVQDIIKLMFDVDSMERTMMEFDL--DMEKMPLGKLSQKQIQSAYKVLTEIYEL  704
                      70        80        90       100       110       120
                 ....*....|....*....|....*....|....*....|....*....|....*....|
consensus     59 ISDGGSPAKLIDLSNRFYTLIPHDFGFKKPP--LIDTHQKIQAKRQMLDALK-EIEVAYS 115
3PAX          67 VSDGGSESQLLDLSNRFYTLIPHDFGMKKPP--LLSNLEYIQAKVQMLDNLL-DIEVAYS 123
gi 1353140   230 KSDGPS---LHDI-NKYYSLIPHSFGFCVPP--KIDSHAKIQAERELLDALKgSIEASLE 283
gi 1709740   344 I-DRYDRTRLEELSGEFYTVIPHDFGFKNMSqfVIDTPQKLKQKIEMVEALG-EIELATK 401
gi 548585    702 LEQGSNTAKLIDATNRFYTLIPHNFGVQLPT--LIETHQQIEDLRQMLDSLA-EIEVAYS 758
gi 1709741   705 IQGGGTNAKFIDATNRFYTLIPHNFGTQSPP--LLDTTEQVEQLRQMLDSLI-EIECAYS 761
                     130
                 ....*....|....*....
consensus    116 LLDLEDTASDKDPLDRHYE 134
3PAX         124 LLRGGNEDGDKDPIDINIE 142
gi 1353140   284 LFDLKNTASSKDIYQRLYE 302
gi 1709740   402 LLSVDPGLQD-DPLYYHYQ 419
gi 548585    759 IIKSEDVSDACNPLDNHYA 777
gi 1709741   762 LLQTEDSKADINPIDKHYE 780
```

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Exhibit J 09/843,159 2/2

NCBI

Conserved Domain Database

CD: pfam00644.8, PARP **PSSM-Id:** 1202 **Source:** Pfam[US], Pfam[UK]

Description: Poly(ADP-ribose) polymerase catalytic domain. Poly(ADP-ribose) polymerase catalyses the covalent attachment of ADP-ribose units from NAD+ to itself and to a limited number of other DNA binding proteins, which decreases their affinity for DNA. Poly(ADP-ribose) polymerase is a regulatory component induced by DNA damage. The carboxyl-terminal region is the most highly conserved region of the protein. Experiments have shown that a carboxyl 40 kDa fragment is still catalytically active.

Taxa: Eukaryota **References:** 3 Pubmed Links

Status: Alignment from source **Created:** 11-Apr-2003

Aligned: 6 rows **PSSM:** 215 columns **Representative:** Consensus

Proteins: [Click here for CDART summary of Proteins containing pfam00644]

View 3D Structure with Cn3D using Virtual Bonds (To display structure, download Cn3D)

View Alignment as Hypertext width 60 color at 2.0 bits

Subset Rows up to 10 of the most diverse members

```
                      10        20        30        40        50        60
                      ....*....|....*....|....*....|....*....|....*....|....*....|
consensus      1 LNCHLEPVDKDSE----EFSILRQYVKNTHASTHNAYDLK---------IVEVFRVSRQG 47
1EFY_A       136 LRTDIKVVDKDSE----EAKIIKQYVKNTHAATHNAYDLK---------VVEIFRIEREG 182
gi_1353140   304 LPCHLEPVSEEIAgkigDCLAMRGPTHCYKLSLIDAFELKdpneiptealPVEVQEVPKKR 363
gi_1709740   421 LNCGLTPVGNDSE----EFSMVANYMENTHAKTHSGYTVE---------IAQLFRASRAV 467
gi_548585    779 IKTQLVALDKNSE----EFSILSQYVKNTHASTHKSYDLK---------IVDVFKVSRQG 825
gi_1709741   782 LKTKLEPLDKNSE----EYILLQKYVKNTHAETHNLYDLE---------VVDIFKVARQG 828

                      70        80        90        100       110       120
                      ....*....|....*....|....*....|....*....|....*....|....*....|
consensus     48 EARRFKPFKKL----HNRRLLWHGSRLTNFAGILSQGLRIAPPEAPVTGYMFGKGVYFAD 103
1EFY_A       183 ESQRYKPFKQL----HNRQLLWHGSRTTNFAGILSQGLRIAPPEAPVTGYMFGKGIYFAD 238
gi_1353140   364 GPKSTKTAAPTvpppTTKRLLWHGTRVTNVFSILMNGLQF--PVGDRCGLMFGNGVYFAN 421
gi_1709740   468 EADRFQQFSSS----HNRMLLWHGSRLTNWAGILSQGLRIAPPEAPVTGYMFGKGVYFAD 523
gi_548585    826 EARRFKPFKKL-----HNRKLLWHGSRLTNFVGILSHGLRIAPPEAPPTGYMFGKGIYFAD 881
gi_1709741   829 EARRYKPFKKL----HNRRLLWHGSRLTNFAGILSHGLKIAPPEAPVTGYMFGKGIYFAD 884

                     130       140       150       160       170       180
                      ....*....|....*....|....*....|....*....|....*....|....*....|
consensus    104 MVSKSANYCQTSQANSTGLMLLCEVALGD---MYELTIAKY-ITKLPNGKHSVKGFGKTA 159
1EFY_A       239 MVSKSANYCHTSQADPIGLILLGEVALGN---MYELKNASH-ITKLPKGKHSVKGLGKTA 294
gi_1353140   422 VFTNSANYC-QPEASKRVFMLLCEVETANplvLYESEIDAD-EKMEKAKKTSVYAAGKHT 479
gi_1709740   524 MFSKSANYCYANTGANDGVLLLCEVALGD---MNELLYSDYnAINLPPGKLSTKGVGKTA 580
gi_548585    882 MVSKSANYCQTSQQNSTGLMLLSEVALGD---MMECTSAKY-INKLSNNKHSCFGRGRTM 937
gi_1709741   885 MVSKSANYCQTSHHNSTGLMLLSEVALGD---MMECTAAKY-VTKLPNDKHSCFGRGRTM 940

                     190       200       210       220       230
                      ....*....|....*....|....*....|....*....|....*....|....*...
consensus    160 PNPTES-ITL-DGVEVPLGNPIETIELKTSLLYNEYIVYNVEQVKIKYVLRVKFNYKT 215
1EFY_A       295 PDPTAT-TTL-DGVEVPLGNGISTGINDTCLLYNEYIVYDVAQVNLKYLLELKFNYKT 350
gi_1353140   480 PRDT---VEI-NGIPAFKSN-LETIEEETRLLYDEYVMPNKEHFKIKYVVEVKVDRLT 532
gi_1709740   591 PNPSEA-QTLeDGVVVPLGKPVERSCSKGMLLYNEYIVYNVEQIKMRYVIQVKFNYKH 647
gi_548585    938 PDPTKSyIRS-DGVEIPiGETITDEHLKSSLLYNEYIVrDVAQVNIQYLFRMEFKYSY 994
gi_1709741   941 PNPSES-IIReDGVEIPLGKPITNDSLKSSLLYNEFIIYDIAQVNIQYMLRMNFKYR- 996
```

Exhibit K 09/843,159

1: Proc Natl Acad Sci U S A. 1996 Jul 23;93(15):7481-5.

Structure of the catalytic fragment of poly(AD-ribose) polymerase from chicken.

Ruf A, Mennissier de Murcia J, de Murcia G, Schulz GE.

Institut fur Organische Chemie und Biochemie, Freiburg im Breisgau, Germany.

The crystal structures of the catalytic fragment of chicken poly(ADP-ribose) polymerase [NAD+ ADP-ribosyltransferase; NAD+:poly(adenosine-diphosphate-D-ribosyl)-acceptor ADP-D-ribosyltransferase, EC 2.4.2.30] with and without a nicotinamide-analogue inhibitor have been elucidated. Because this enzyme is involved in the regulation of DNA repair, its inhibitors are of interest for cancer therapy. The inhibitor shows the nicotinamide site and also suggests the adenosine site. The enzyme is structurally related to bacterial ADP-ribosylating toxins but contains an additional alpha-helical domain that is suggested to relay the activation signal issued on binding to damaged DNA.

PMID: 8755499 [PubMed - indexed for MEDLINE]

Exhibit L 09/843,159

1: Gene. 1993 Dec 31;137(2):293-7. Related Articles, Links

Isolation of the poly(ADP-ribose) polymerase-encoding cDNA from Xenopus laevis: phylogenetic conservation of the functional domains.

Uchida K, Uchida M, Hanai S, Ozawa Y, Ami Y, Kushida S, Miwa M.

Department of Biochemistry, University of Tsukuba, Japan.

The complete nucleotide (nt) sequence of the Xenopus laevis poly(ADP-ribose) polymerase (PARP)-encoding cDNA was determined. The putative X. laevis PARP protein consists of 1008 amino acids (aa) with a molecular weight of 113 kDa. X. laevis PARP shares 74, 83, 73, 78 and 42% aa sequence homology with the human, bovine, mouse, chicken and Drosophila melanogaster PARPs, respectively. Comparison of the PARP aa sequences among these species showed conservation of two zinc-finger motifs in the DNA-binding domain, and an NAD-binding motif and a Rossmann fold in the catalytic domain. The first Leu of the putative leucine zipper of D. melanogaster PARP is substituted to Lys in X. laevis PARP. All the Glu residues in the leucine zipper are conserved in these six species.

PMID: 8299962 [PubMed - indexed for MEDLINE]

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1: Biochimie. 1995;77(6):456-61. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Poly(ADP-ribose) polymerase: structure-function relationship.

Masson M, Rolli V, Dantzer F, Trucco C, Schreiber V, Fribourg S, Molinete M, Ruf A, Miranda EA, Niedergang C, et al.

Ecole Superieure de Biotechnologie de Strasbourg, UPR 9003 du CNRS, Illkirch, France.

Dissection of the human poly(ADP-ribose) polymerase (PARP) molecule in terms of its structure-function relationship has proved to be an essential step towards understanding the biological role of poly(ADP-ribosylation) as a cellular response to DNA damage in eukaryotes. Current approaches aimed at elucidating the implication of this multifunctional enzyme in the maintenance of the genomic integrity will be presented.

PMID: 7578429 [PubMed - indexed for MEDLINE]

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1: Mol Cell Biochem. 1994 Sep;138(1-2):15-24. Related Articles, Links

Structure and function of poly(ADP-ribose) polymerase.

de Murcia G, Schreiber V, Molinete M, Saulier B, Poch O, Masson M, Niedergang C, Menissier de Murcia J.

Ecole Superieure de Biotechnologie de Strasbourg, Unite de Cancerogenese et de Mutagenese Moleculaire et Structurale, Centre National de la Recherche Scientifique, Illkirch-Graffenstaden, France.

Poly(ADP-ribose) polymerase (PARP) participates in the intricate network of systems developed by the eukaryotic cell to cope with the numerous environmental and endogenous genetoxic agents. Cloning of the PARP gene has allowed the development of genetic and molecular approaches to elucidate the structure and the function of this abundant and highly conserved enzyme. This article summarizes our present knowledge in this field.

Publication Types:
- Review
- Review, Tutorial

PMID: 7898458 [PubMed - indexed for MEDLINE]

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Exh. 6.6 O 09/863,159

1: J Biol Chem. 1993 Apr 25;268(12):8529-35. Related Articles, Links

Identification of potential active-site residues in the human poly(ADP-ribose) polymerase.

Simonin F, Poch O, Delarue M, de Murcia G.

Unite propre de recherche de Cancerogenese et de Mutagenese Moleculaire et Structurale, Centre National de la Recherche Scientifique, Strasbourg, France.

The carboxyl-terminal catalytic domain of the human poly(ADP-ribose) polymerase (PARP) exhibits sequence homology with the NAD(P)(+)-dependent leucine and glutamate dehydrogenases. To clarify the role played by some conserved residues between PARP and NAD(P)(+)-dependent dehydrogenases, point mutations were introduced into the whole enzyme context. Non-conservative mutations of Lys-893 (K893I) and Asp-993 (D993A) completely inactivate human PARP, whereas conservative and nonconservative mutations of Asp-914 (D914E and D914A, respectively) and Lys-953 (K953R and K953I, respectively) partially alter PARP activity. The consequences of conservative substitution of Lys-893 and Asp-993 on the kinetic properties of human poly(ADP-ribose) polymerase enzyme and the polymer it synthesizes suggest that these 2 amino acids are directly involved in the covalent attachment of the first ADP-ribosyl residue from NAD+ onto the acceptor amino acid. In addition, the recent resolution of the three-dimensional structure of the NAD(+)-linked glutamate dehydrogenase from Clostridium symbiosum (Baker, P.J., Britton, K.L., Engel, P.C., Farrants, G.W., Lilley, K.S., Rice, D.W., and Stillman, T.J. (1992) Proteins 12, 75-86) strongly supports our alignment with leucine and glutamate dehydrogenases and provides an interesting structural framework for the analysis of our results of site-directed mutagenesis.

PMID: 8473297 [PubMed - indexed for MEDLINE]

Exhibit P 09/843,159

1: Biochemistry. 1997 Oct 7;36(40):12147-54.

ACS PUBLICATIONS

Random mutagenesis of the poly(ADP-ribose) polymerase catalytic domain reveals amino acids involved in polymer branching.

Rolli V, O'Farrell M, Menissier-de Murcia J, de Murcia G.

Ecole Superieure de Biotechnologie de Strasbourg, UPR A9003 du CNRS, Illkirch-Graffenstaden, France.

Poly(ADP-ribose) polymerase (PARP) is a multifunctional nuclear zinc finger protein which participates in the immediate response of mammalian cells exposed to DNA damaging agents. Given the complexity of the poly(ADP-ribosylation) reaction, we developed a large-scale screening procedure in Escherichia coli to identify randomly amino acids involved in the various aspects of this mechanism. Random mutations were generated by the polymerase chain reaction in a cDNA sequence covering most of the catalytic domain. Out of 26 individual mutations that diversely inactivated the full-length PARP, 22 were found at conserved positions in the primary structure, and 24 were located in the core domain formed by two beta-sheets containing the active site. Most of the PARP mutants were altered in poly(ADP-ribose) elongation and/or branching. The spatial proximity of some residues involved in chain elongation (E988) and branching (Y986) suggests a proximity or a superposition of these two catalytic sites. Other residues affected in branching were located at the surface of the molecule (R847, E923, G972), indicating that protein-protein contacts are necessary for optimal polymer branching. This screening procedure provides a simple and efficient method to explore further the structure-function relationship of the enzyme.

PMID: 9315851 [PubMed - indexed for MEDLINE]